PAPILLOMAVIRUS E2 PROTEIN IS REGULATED BY SPECIFIC FIBROBLAST GROWTH FACTOR RECEPTORS

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Abstract

The papillomavirus (PV) E2 protein activates transcription and replication by recruiting cellular proteins and the E1 DNA helicase to their binding sites in the viral genome. We recently demonstrated that phosphorylation of tyrosine 102 in the bovine papillomavirus (BPV-1) E2 protein restricts these activities and that fibroblast growth factor receptor-3 (FGFR3) tyrosine kinase binds PV E2. Expression of FGFR3 decreased viral replication with both wild-type and the phenylalanine substitution at position 102, inferring that another kinase targets Y102. Here we tested FGFR- 1, -2 and -4 for association with PV E2 proteins. FGFR2 but not FGFR1 or FGFR4 co-immunoprecipitated with BPV-1 E2. We found that FGFR2 suppressed replication but did not depend on phosphorylation of BPV-1 Y102. HPV-16 and -31 E2 interacted with FGFR1, -2, and -4. These results imply that the expression and activity of FGF receptors in epithelial cells can regulate the function of E2 in viral replication.

Keywords

Papillomavirus E2 phosphorylation, viral replication, Fibroblast growth factor receptor

Highlights

- BPV and HPV E2 proteins interact with different Fibroblast Growth Factor Receptors
- Over-expression of FGFR increased E2 tyrosine phosphorylation and reduced HPV DNA replication, though not mediated through tyrosine 102
- Mass spectrometry identified BPV E2 tyrosine phosphorylations.

Introduction

Papillomaviruses infect stratifying epithelia that are programmed to undergo terminal differentiation. Upon entry into basal cells, a single closed circular double stranded viral episome replicates to perhaps 10-50 copies per cell. This stage depends on host replicative factors; cellular and viral proteins are not packaged within the virion. Despite this initial amplification, PVs only express trace amounts of viral proteins and do not cause lytic infections. The E1 and E2 proteins are required for PV replication and mutations that render these inactive eventuate in either integration into stochastic chromosomal locations or loss of viral DNA [(Schiller et al., 1989), reviewed in (Kadaja et al., 2009)].

The E2 protein binds directly to and recruits the E1 DNA helicase to their recognition motifs that form the viral *origin* of replication (Androphy et al., 1987; Mohr et al., 1990). E1 monomers assemble into active double hexamers, which requires release from the E2 protein (Sanders and Stenlund, 1998). Several post-translational modifications of E2 protein are known to regulate its activities. We recently reported the acetylation of the bovine papillomavirus (BPV-1) E2 protein at lysine (K) 111 and K112 (Quinlan et al., 2013) and HPV-31 E2 at K111, and that this was necessary for unwinding of the replication fork (Thomas and Androphy, 2018). Phosphorylation of E2 on specific serine and threonine residues are known to affect its stability, chromatin binding and protein-protein interactions [(Chang et al., 2014), reviewed in (McBride, 2013)]. Recently, we detected tyrosine (Y) phosphorylation of BPV-1 E2 at amino acid 102 and that the phosphomimetic glutamate substitution reduced E2 transcription and replication activity (Culleton et al., 2017). Subsequently, fibroblast growth factor receptor-3 (FGFR3) was found to induce phosphorylation of tyrosine that restricts PV genome replication, although this was not mediated through Y102 (Xie et al., 2017).

Fibroblast growth factor receptors (FGFRs) are a group of four transmembrane tyrosine kinase receptors with multiple isoforms (Gong, 2014). The FGF signaling pathway regulates

multiple biological processes such as angiogenesis, and tissue development and regeneration (Touat et al., 2015). FGFRs are involved in varying stages of viral infections. For example, FGFR1 may be a co-receptor for adeno-associated virus (AAV) 2 (Qing et al., 1999) and AAV-3 (Blackburn et al., 2006). FGFR1 suppresses influenza virus replication (Liu et al., 2015) and is activated by Epstein Barr Virus protein latent membrane protein 1 (LMP1) facilitating epithelial cell transformation (Lo et al., 2015). FGFR4 is involved in infectivity of a modified Influenza virus (Konig et al., 2010). FGFR1 and FGFR4 expression were increased in long-term Kaposi's sarcoma-associated herpesvirus (KSHV) infected telomerase-immortalized human umbilical vein endothelial cells (An et al., 2006). HPV-16 E5 protein targeting of FGFR2 inhibits autophagy, possibly affecting the early stages of HPV infection (Belleudi et al., 2015).

In a search for tyrosine kinases in complex with the E2 protein, an activated FGFR3 mutant was shown to suppress transient viral DNA replication (Xie et al., 2017). However, this did not require phosphorylation of E2 at Y102, inferring that another tyrosine kinase might target this residue and that other phosphotyrosines could be mediating the inhibitory effect of FGFR3. Our next goal was to determine if another FGFR family member complexes with and regulates E2 function. We found that only FGFR2 interacted with BPV-1 E2 while FGFR-1, -2, and -4 complex with HPV-16 and HPV-31 E2. However only endogenous FGFR-2 could be co-immunoprecipitated (co-ip'd) with HPV-16 E2.

Materials and Methods

Plasmids and antibodies

Codon optimized FLAG HPV-31 E2 (DeSmet et al., 2016) and the ori-luciferase plasmids for the PV transient replication assay (Fradet-Turcotte et al., 2010) were used as previously reported (DeSmet et al., 2016). FGFR-1, 2, and 4 constructs were provided by L. Thompson (UC Irvine). A Myc tag was added to the C terminus of FGFRs by PCR amplification using the following primers Bam-FGFR1-F: GATCGGATCCATGTGGAGCT, FGFR1-myc-Not-R:

GTACGCGGCCGCTCACAGATCCTCTTCTGAGATGAGTTTTTGTTCGCGGCGTTT, Bam-FGFR2-F: GATCGGATCCATGGTCAGCT, FGFR2-myc-Not-R: GTACGCGGCCGCTCACAGATCCTCTTCTGAGATGAGTTTTTGTTCTGTTTTAACACTG, Bam-FGFR3-F: GATCGGATCCATGGGCGCCCCT, FGFR3-myc-Not-R: GTACGCGGCCGCTCACAGATCCTCTTCTGAGATGAGTTTTTGTTCCGTCCGCGA, Kpn-FGFR4-F: GATCGGTACCATGCGGCTGC, FGFR4-myc-Not-R: GTACGCGGCCGCTCACAGATCCTCTTCTGAGATGAGTTTTTGTTCTGTCTGCAC, and inserted into pcDNA3. The following antibodies were used: mouse anti-FLAG M2, phospho-Tyrosine specific PY-99 (Santa Cruz) and PY-100 (Cell Signaling), rabbit anti-MYC (Cell Signaling), anti-FGFR1 (Abcam), anti-FGFR2 (Santa Cruz), and anti-FGFR4 (Santa Cruz). BPV-1 E2 was identified with B201, a mouse monoclonal antibody with an epitope between amino acids (aa) 160-220 (Breiding et al., 1996). Mouse-anti HPV-16-E2 (TVG-261) and HPV-16 E2 sheep-antiserum (Siddiqa et al., 2015) were used to identify HPV E2 proteins.

Cell Culture

All cell lines were maintained at 37˚C and 5% CO_2. HEK293TT (from J. Schiller and C. Buck) and C33A (from D. Lowy) were cultured in Dulbecco's Modified Eagle Medium (Life Technologies) with 10% fetal bovine serum (Peak Serum) and penicillin/streptomycin (100 U/ml; Life Technologies). CIN612-9E (from Laimonis Laimins) a clonal cell line that maintains HPV-31 genomes were grown in E-medium with J23T3 fibroblast feeders (from Howard Green). W12 (from M. Stanley and P. Lambert) that maintain HPV-16 episomes were grown in F medium with J23T3 fibroblast feeders. NOKS (from I. Morgan) were grown in Keratinocyte-SFM containing human recombinant Epidermal Growth Factor 1-53, Bovine Pituitary Extract (BPE) and penicillin/streptomycin (100 U/ml; Life Technologies).

In-situ proximity ligation assay (PLA)

In-situ PLA was performed using the PLA Red kit following Olink Biosciences' instructions. CIN612 cells were transfected with FLAG-HPV-31 E2. NOKS cells were transfected with FLAG-HPV-31 E2 and FGFR2. 24 h later, cells were fixed in 4% paraformaldehyde for 10 min, permeabilized for 15 min in 0.5% Triton-X 100/PBS, washed in PBS, blocked with 5% goat serum in 0.2% Triton-X 100/PBS, then incubated overnight with primary antibody combinations (mouse M2 FLAG-HPV-31 E2 or rabbit FGFR2) at 4 °C.

Co-immunoprecipitations and Immunoblotting

Cells were transfected using Lipofectamine 2000 (Life Technologies) according to manufacturers' instructions or with polyethylenimine (PEI) (2 mg/ml) (1 µg DNA: 2 mg PEI). After 24-48 hrs., cells were lysed in 0.5% NP-40 containing 150 mM NaCl, 20 mM HEPES, pH 7.4, 1 mM Na_3VO_4 and protease inhibitor cocktail (Sigma). To each reaction, 30 µl of 50% protein A/G slurry (Invitrogen) along with 1 µg of antibody or FLAG epitope specific M2 beads (Sigma) were added and rotated overnight at 4˚C. Beads were washed 3 times in lysis buffer. Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto PVDF membranes (Millipore), blocked in 5% Tris buffered saline (TBS)-Tween (0.1%) BSA, and probed with specific antibodies and corresponding secondary antibodies. Chemiluminescence substrates (Thermo Scientific) were used to detect horse raddish peroxidase conjugated secondary antibody signal. For endogenous HPV-16 E2 co-immunoprecipitation, W12 cells were grown to 80% confluence. Cells were lysed in 50 mM HEPES [pH 7.4], 150 mM NaCl, 1 mM Na_3VO_4, 0.5% NP-40, 1 mM DTT, and 1X protease inhibitor cocktail. Sheep-anti-HPV16-E2 polyclonal sera or sheep nonspecific IgG (Santa Cruz) were added to the lysates and rotated overnight at 4˚C. Immunoprecipitates were collected using protein A/G slurry for 3h at 4˚C. Beads were washed with lysis buffer and with high salt buffer (50 mM HEPES [pH 7.4], 500 mM NaCl, 1 mM Na_3VO_4, 0.5% NP-40, 1 mM DTT, and 1X protease inhibitor cocktail). Proteins were removed from beads with 2X SDS lysis buffer,

separated by 4-12% SDS-PAGE, and detected by western blot using anti-FGFR and anti-HPV16 E2 (TVG-261) antibodies.

Luciferase DNA Replication Assays

C33A cells (0.5% FBS DMEM) were seeded into a 96 well plate and each well was transfected with 100 or 200 ng FGFR constructs, 0.5 ng pRL (Rluc), 2.5 ng pFLORIBPV-1 or pFLORI31, 10 ng pCG-BPV-1 Eag1235 E1 or codon optimized triple FLAG-HPV-31-E1 (Fradet-Turcotte et al., 2010) and 10 ng pCG-E2 or pSG5-HA-HPV-31 E2 using Lipofectamine 2000. 72 h later cells were lysed and luciferase activity was measured using Dual Glo (Promega). Firefly luciferase levels were normalized to renilla luciferase levels.

Luciferase PV Transcriptional Assays

C33A cells (0.5% FBS DMEM) were seeded into a 96 well plate and each well was transfected with 100 ng FGFR2, 10 ng pCG-BPV-1 E2 and 75 ng pGL2-E2BS-Luc (Kumar et al., 2007) using Lipofectamine 2000. 48 h later cells were lysed and Firefly luciferase activity was measured using Dual Glo (Promega).

Mass spectrometry

293TT cells in 6x15cm plates were transfected with BPV-1 E2 or FGFR2 constructs using PEI for 48hrs. Cells were washed in HBSS buffer (10 mM HEPES pH 7.5, 140 mM NaCl, 0.5 mM $CaCl_2$, 0.8 mM $MgCl_2$), and incubated with the irreversible tyrosine phosphatase inhibitor pervanadate (30 μM) for 1hr at 37˚C. Cells were lysed in 20 mM HEPES pH 7.5, 150 mM NaCl, 30 μM pervanadate, 7.5 μM Trichostatin A (TSA), and 0.525 mg/mL NaF and immunoprecipitated overnight with B201 antibody. Bands were excised from Coomassie stained polyacrylamide gels for tandem mass spectrometry. The gel bands were subjected to reduction (10 mM DTT) and alkylation (55 mM iodoacetamide) and digested with trypsin (Promega) overnight at 37°C and injected into a C18 column. Peptide spectra were recorded in Orbitrap Velos Pro (Thermo-Fisher Scientific) and Dionex UltiMate 3000 RSLC nanoflow system

(Thermo) and database searches were carried out using Sequest algorithm. From the MS/MS data, individual probability values for each phosphorylation site was calculated using Proteome Discoverer V1.3 equipped with phospho RS 2.0.

Statistical Analysis

Each experiment was repeated at least three times. Two-way or one-way t-test was used for analysis. Means are expressed +/- SEM. * indicates p-values ≤ 0.05.

Results

FGFR2 but not FGFR1 nor FGFR4 interact with BPV-1 E2 protein and impairs viral replication.

After observing an interaction between FGFR3 and BPV-1 E2, we explored whether other FGFRs interacted with BPV-1 E2. HEK293TT cells were transfected with FGFR- 1, 2 and 4 expression vectors along with BPV-1 E2 and the truncated form BPV-1 E2R (aa 162-410). BPV-1 E2 full length increases the transcription of genes co-transfected, unlike E2R (Haugen et al., 1987). Full length E2 but not the E2R protein co-immunoprecipitated with FGFR2 while FGFR4 or FGFR1 did not (Fig. 1A-C). Heterologous FGFR2 increased tyrosine phosphorylation of wild type (wt) BPV-1 E2 (Fig. 1D). The multiple bands detected for FGFR proteins may be spliced variants (Gong, 2014). Furthermore, total phosphotyrosine of BPV-1 E2 the phenylalanine (F) mutation Y102F still approximated that of wild type after FGFR2 transfection (Fig. 1D).

Because BPV-1 E2 bound FGFR2 strongly, the effect of the FGFR kinase activity on E2 and E1 dependent BPV-1 DNA replication was evaluated using a luciferase reporter based system (Fradet-Turcotte et al., 2010). C33A cells were co-transfected with BPV-1 E1 and E2 expression vectors along with a BPV-1 luciferase linked reporter and the FGFR1, 2, and 4 expression constructs. All the FGFR constructs demonstrated capability to induce tyrosine phosphorylations (Fig. 2A). FGFR2 over-expression reduced BPV-1 transient replication while FGFR1 and FGFR4 had no effect (Fig. 2B). To determine if Y102 may be a target of FGFR2, replication

assays were performed with BPV-1 E2 Y102F in the presence of FGFR2 together with E1. If the Y102 residue was a direct target for FGFR2, the Y102F mutant should not be inhibited by FGFR2. Consistent with our previous study (Culleton et al., 2017), Y102F displayed enhanced transient replication compared to wild-type (Fig. 2C). However, these experiments showed that FGFR2 suppressed Y102F mediated replication (Fig. 2C). FGFR2 over-expression had no effect on BPV-1 E2 transcriptional activation (Fig. 2D).

FGFR2 phosphorylates E2 at tyrosine residues.

To determine the E2 residues phosphorylated by FGFR2, HEK293TT cells expressing BPV-1 E2 with and without FGFR2 were analyzed by mass spectrometry. We obtained 91.95% (wt-E2) and 93.41% (E2+FGFR2) sequence coverage for the samples and found that BPV-1 E2 was phosphorylated at the conserved tyrosine residues 32, 131, 158, and 159 with FGFR2 over-expression. We also detected phosphotyrosine at the non-conserved residues Y44, Y169, Y170, and Y262 (Fig. 2E). Phosphorylation at tyrosine 195 was detected both with and without FGFR2 over-expression, suggesting this may not be FGFR2 dependent.

HPV-16 and HPV-31 E2 bind FGFR family proteins

Our next goal was to characterize the interactions between HPV-16 and HPV-31 E2 and the FGFR proteins. For direct comparison, FGFR-1, -2 and -4 were each Myc epitope tagged and co-transfected with FLAG-HPV-16 E2 into HEK293TT cells. HPV-16 E2 co-ip'd with FGFR1,-2, and -4 (Fig. 3A). To address whether endogenous levels of each FGFR and HPV-16 E2 proteins are in complex, W12 cell lysates were immunoprecipitated using an antibody for HPV-16 E2 (Siddiqa et al., 2015). These experiments demonstrated HPV-16 E2 in association with FGFR2, while FGFR1 and FGFR4 (Fig. 3B) were not, despite their confirmed expression in the input lanes. Analogous transfections showed HPV-31 E2 also co-ip'd with all these Myc-tagged FGFRs (Fig. 3C). Because our HPV-31 E2 antibody does not recognize the endogenous E2

protein by Western blot, to further support this association, we utilized PLA to determine whether endogenous FGFR2 was within molecular proximity of transfected FLAG-HPV-31 E2 using the cervical dysplasia derived CIN612 cell line that maintains multi-copy HPV-31 episomes and using NOKS, oral keratinocytes without HPV. Multiple interaction foci were detected in the nucleus but not cytoplasm, confirming our observation that FGFR2 and E2 are in complexes *in vivo* (Fig. 3D). We did not observe any PLA foci in untransfected cells using FGFR2 and FLAG antibodies. Because of their high background in immunofluorescence experiments, we could not interpret PLA experiments using FGFR1 or 4 antibodies with E2. Over-expression of FGFR2 increased tyrosine phosphorylation of HPV-31 E2 as detected using a phosphotyrosine specific antibody (Fig. 3E). This FGFR2 activity also impaired HPV-31 transient replication in the reporter assay as observed for BPV-1 transient replication (Fig. 4A) but showed no alteration of HPV-31 E1 protein levels (Fig. 4B).

Discussion

We recently detected phosphorylation of tyrosine Y102 in BPV-1 E2 and reported that FGFR3 binds to E2 and limits E1 dependent viral DNA replication (Culleton et al., 2017; Xie et al., 2017). In the current study, we addressed the potential role of other FGFRs in regulating the replication functions of bovine and human PV E2. We observed the interaction between FGFR2 and E2 by co-immunoprecipitation and PLA and demonstrated that FGFR2 phosphorylates tyrosine residues in BPV E2 that results in decreased PV DNA replication. To identify which FGFR may interact with HPV E2, these were Myc-epitope tagged and co-transfected along with HPV-16 and HPV-31 E2. While HPV-16 and HPV-31 E2 co-ip'd all the Myc-FGFRs, FGFR2 and FGFR4 were strongest respectively, yet only endogenous FGFR2 could be co-ip'd with HPV-16 E2. Endogenous FGFR-3 also complexes with HPV-16 E2 (Xie et al., 2017). Further, over-expression of FGFR2 suppressed BPV-1 and HPV-31 replication, indicating that FGFR2 and FGFR3 (Xie et al., 2017) are the most likely tyrosine kinases that phosphorylated E2 protein *in*

vivo. Several FGFR inhibitors are commercially available, however these are not FGF receptor type specific and have off target effects (Katoh, 2016). We tested several FGFR inhibitors but the data were not robust as these also inhibited cell proliferation.

Bioinformatic analysis to identify a potential kinase and target tyrosine residues of E2 has been challenging. Most of the predictive tools have given inconsistent results; hence we approached the problem by directly studying the interaction between E2 protein and the FGF family of receptors. Our results are consistent with the expression profiles of these kinases. Human protein atlas (HPA) data base analysis suggests high expression of FGFR2 (97.5 transcripts per million – TPM) when compared to FGFR1 (17.7 TPM) or FGFR4 (1.1 TPM) in skin tissue samples (Uhlen et al., 2005; Uhlen et al., 2015; Uhlen et al., 2010). Only FGFR3 has higher expression (334.6 TPM) than FGFR2. It is likely that PV E2 interacts with and is regulated by these relatively abundant skin tissue tyrosine kinases. Interestingly, the median expression of FGFR2 and FGFR3 across several skin tissues is reduced in primary tumor samples compared to normal samples as analyzed using Metabolic gEne Rapid Visualizer (Shaul et al., 2016), suggesting growth inhibitory properties for these two kinases.

In an effort to identify the tyrosine residues phosphorylated by FGFR2, we performed mass spectrometry with immunoprecipitated BPV-1 E2 protein from 293TT cells. The majority of the tyrosine phosphorylations detected were located throughout the transactivation domain (at amino acids 32, 44, 131, 158, 159, 169, and 170), suggesting these may influence protein-protein interactions. For example, HPV-18's Y36 (homologous to BPV Y32) in the E2/E1 co-crystal structure (PDB 1TUE) (Abbate et al., 2004) directly faces E1, and therefore could disrupt binding. Similarly HPV16 Y44 in the E2/Brd4 co-crystal structure (PDB 2NNU) (Abbate et al., 2006) is oriented externally towards the Brd4 fragment. Residue Y131 mediates binding to the cellular protein Chlr1. Phosphorylation at this residue could disrupt this protein-protein interaction, which is required for efficient viral genome segregation and maintenance in cells

(Harris et al., 2017). We speculate that at least some if not all phosphorylations among these are inhibitory to viral replication and are specifically induced by FGFR2 and FGFR3. Due to the qualitative nature of mass spectrometry, the lack of detected phospho-tyrosines at other positions does not exclude the possibility of a phosphorylation. Future studies are necessary to characterize and validate each of these detected tyrosine residues as potential phosphorylation sites for FGFR2.

In this study, we provide another novel interaction partner for PV E2 and conclude that FGFR2 and FGFR3 phosphorylate E2 to regulate viral replication. Both FGFR2 and FGFR3 are highly expressed in keratinocytes. Further experiments are necessary to determine the tyrosines modified by these kinases in epithelial cells. Mutational analysis of these HPV E2 residues should provide mechanistic insights into the viral lifecycle.

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References

Abbate, E.A., Berger, J.M., Botchan, M.R., 2004. The X-ray structure of the papillomavirus helicase in complex with its molecular matchmaker E2. Genes & development 18, 1981-1996.

Abbate, E.A., Voitenleitner, C., Botchan, M.R., 2006. Structure of the papillomavirus DNA-tethering complex E2:Brd4 and a peptide that ablates HPV chromosomal association. Molecular cell 24, 877-889.

An, F.Q., Folarin, H.M., Compitello, N., Roth, J., Gerson, S.L., McCrae, K.R., Fakhari, F.D., Dittmer, D.P., Renne, R., 2006. Long-term-infected telomerase-immortalized endothelial cells: a model for Kaposi's sarcoma-associated herpesvirus latency in vitro and in vivo. Journal of virology 80, 4833-4846.

Androphy, E.J., Lowy, D.R., Schiller, J.T., 1987. Bovine papillomavirus E2 trans-activating gene product binds to specific sites in papillomavirus DNA. Nature 325, 70-73.

Belleudi, F., Nanni, M., Raffa, S., Torrisi, M.R., 2015. HPV16 E5 deregulates the autophagic process in human keratinocytes. Oncotarget 6, 9370-9386.

Blackburn, S.D., Steadman, R.A., Johnson, F.B., 2006. Attachment of adeno-associated virus type 3H to fibroblast growth factor receptor 1. Archives of virology 151, 617-623.

Breiding, D.E., Grossel, M.J., Androphy, E.J., 1996. Genetic analysis of the bovine papillomavirus E2 transcriptional activation domain. Virology 221, 34-43.

Chang, S.W., Liu, W.C., Liao, K.Y., Tsao, Y.P., Hsu, P.H., Chen, S.L., 2014. Phosphorylation of HPV-16 E2 at serine 243 enables binding to Brd4 and mitotic chromosomes. PloS one 9, e110882.

Culleton, S.P., Kanginakudru, S., DeSmet, M., Gilson, T., Xie, F., Wu, S.Y., Chiang, C.M., Qi, G., Wang, M., Androphy, E.J., 2017. Phosphorylation of the Bovine Papillomavirus E2 Protein on Tyrosine Regulates Its Transcription and Replication Functions. Journal of virology 91, e01854-16.

DeSmet, M., Kanginakudru, S., Rietz, A., Wu, W.H., Roden, R., Androphy, E.J., 2016. The Replicative Consequences of Papillomavirus E2 Protein Binding to the Origin Replication Factor ORC2. PLoS pathogens 12, e1005934.

Fradet-Turcotte, A., Morin, G., Lehoux, M., Bullock, P.A., Archambault, J., 2010. Development of quantitative and high-throughput assays of polyomavirus and papillomavirus DNA replication. Virology 399, 65-76.

Gong, S.G., 2014. Isoforms of receptors of fibroblast growth factors. Journal of cellular physiology 229, 1887-1895.

Harris, L., McFarlane-Majeed, L., Campos-Leon, K., Roberts, S., Parish, J.L., 2017. The Cellular DNA Helicase ChlR1 Regulates Chromatin and Nuclear Matrix Attachment of the Human Papillomavirus 16 E2 Protein and High-Copy-Number Viral Genome Establishment. Journal of virology 91, e01853-16.

Haugen, T.H., Cripe, T.P., Ginder, G.D., Karin, M., Turek, L.P., 1987. Trans-activation of an upstream early gene promoter of bovine papilloma virus-1 by a product of the viral E2 gene. The EMBO journal 6, 145-152.

Kadaja, M., Silla, T., Ustav, E., Ustav, M., 2009. Papillomavirus DNA replication - from initiation to genomic instability. Virology 384, 360-368.

Katoh, M., 2016. FGFR inhibitors: Effects on cancer cells, tumor microenvironment and whole-body homeostasis (Review). International journal of molecular medicine 38, 3-15.

Konig, R., Stertz, S., Zhou, Y., Inoue, A., Hoffmann, H.H., Bhattacharyya, S., Alamares, J.G., Tscherne, D.M., Ortigoza, M.B., Liang, Y., Gao, Q., Andrews, S.E., Bandyopadhyay, S., De Jesus, P., Tu, B.P., Pache, L., Shih, C., Orth, A., Bonamy, G., Miraglia, L., Ideker, T., Garcia-Sastre, A., Young, J.A., Palese, P., Shaw, M.L., Chanda, S.K., 2010. Human host factors required for influenza virus replication. Nature 463, 813-817.

Kumar, R.A., Naidu, S.R., Wang, X., Imbalzano, A.N., Androphy, E.J., 2007. Interaction of papillomavirus E2 protein with the Brm chromatin remodeling complex leads to enhanced transcriptional activation. Journal of virology 81, 2213-2220.

Liu, X., Lai, C., Wang, K., Xing, L., Yang, P., Duan, Q., Wang, X., 2015. A Functional Role of Fibroblast Growth Factor Receptor 1 (FGFR1) in the Suppression of Influenza A Virus Replication. PloS one 10, e0124651.

Lo, A.K., Dawson, C.W., Young, L.S., Ko, C.W., Hau, P.M., Lo, K.W., 2015. Activation of the FGFR1 signalling pathway by the Epstein-Barr virus-encoded LMP1 promotes aerobic glycolysis and transformation of human nasopharyngeal epithelial cells. The Journal of pathology 237, 238-248.

McBride, A.A., 2013. The papillomavirus E2 proteins. Virology 445, 57-79.

Mohr, I.J., Clark, R., Sun, S., Androphy, E.J., MacPherson, P., Botchan, M.R., 1990. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. Science 250, 1694-1699.

Qing, K., Mah, C., Hansen, J., Zhou, S., Dwarki, V., Srivastava, A., 1999. Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. Nature medicine 5, 71-77.

Quinlan, E.J., Culleton, S.P., Wu, S.Y., Chiang, C.M., Androphy, E.J., 2013. Acetylation of conserved lysines in bovine papillomavirus E2 by p300. Journal of virology 87, 1497-1507.

Sanders, C.M., Stenlund, A., 1998. Recruitment and loading of the E1 initiator protein: an ATP-dependent process catalysed by a transcription factor. The EMBO journal 17, 7044-7055.

Schiller, J.T., Kleiner, E., Androphy, E.J., Lowy, D.R., Pfister, H., 1989. Identification of bovine papillomavirus E1 mutants with increased transforming and transcriptional activity. Journal of virology 63, 1775-1782.

Shaul, Y.D., Yuan, B., Thiru, P., Nutter-Upham, A., McCallum, S., Lanzkron, C., Bell, G.W., Sabatini, D.M., 2016. MERAV: a tool for comparing gene expression across human tissues and cell types. Nucleic acids research 44, D560-566.

Siddiqa, A., Leon, K.C., James, C.D., Bhatti, M.F., Roberts, S., Parish, J.L., 2015. The human papillomavirus type 16 L1 protein directly interacts with E2 and enhances E2-dependent replication and transcription activation. The Journal of general virology 96, 2274-2285.

Thomas, Y., Androphy, E.J., 2018. Human Papillomavirus Replication Regulation by Acetylation of a Conserved Lysine in the E2 Protein. Journal of virology 92, 01912-17.

Touat, M., Ileana, E., Postel-Vinay, S., Andre, F., Soria, J.C., 2015. Targeting FGFR Signaling in Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 21, 2684-2694.

Uhlen, M., Bjorling, E., Agaton, C., Szigyarto, C.A., Amini, B., Andersen, E., Andersson, A.C., Angelidou, P., Asplund, A., Asplund, C., Berglund, L., Bergstrom, K., Brumer, H., Cerjan, D., Ekstrom, M., Elobeid, A., Eriksson, C., Fagerberg, L., Falk, R., Fall, J., Forsberg, M., Bjorklund, M.G., Gumbel, K., Halimi, A., Hallin, I., Hamsten, C., Hansson, M., Hedhammar, M., Hercules, G., Kampf, C., Larsson, K., Lindskog, M., Lodewyckx, W., Lund, J., Lundeberg, J., Magnusson, K., Malm, E., Nilsson, P., Odling, J., Oksvold, P., Olsson, I., Oster, E., Ottosson, J., Paavilainen, L., Persson, A., Rimini, R., Rockberg, J., Runeson, M., Sivertsson, A., Skollermo, A., Steen, J., Stenvall, M., Sterky, F., Stromberg, S., Sundberg, M., Tegel, H., Tourle, S., Wahlund, E., Walden, A., Wan, J., Wernerus, H., Westberg, J., Wester, K., Wrethagen, U., Xu, L.L., Hober, S., Ponten, F., 2005. A human protein atlas for normal and cancer tissues based on antibody proteomics. Molecular & cellular proteomics 4, 1920-1932.

Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C.A., Odeberg, J., Djureinovic, D., Takanen, J.O., Hober, S., Alm, T., Edqvist, P.H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., von

Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., Ponten, F., 2015. Proteomics. Tissue-based map of the human proteome. Science 347, 1260419.

Uhlen, M., Oksvold, P., Fagerberg, L., Lundberg, E., Jonasson, K., Forsberg, M., Zwahlen, M., Kampf, C., Wester, K., Hober, S., Wernerus, H., Bjorling, L., Ponten, F., 2010. Towards a knowledge-based Human Protein Atlas. Nature biotechnology 28, 1248-1250.

Xie, F., DeSmet, M., Kanginakudru, S., Jose, L., Culleton, S.P., Gilson, T., Li, C., Androphy, E.J., 2017. Kinase Activity of Fibroblast Growth Factor Receptor 3 Regulates Activity of the Papillomavirus E2 Protein. Journal of virology 91, e01066-17.

Figure Legends

Fig. 1: FGFR2 but not FGFR4 and FGFR1 interacts with BPV-1 E2. (A) HEK293TT cells were transfected with FGFR2 and BPV-1 full-length or E2R (aa 162-410) constructs. BPV-1 E2 protein pull-down was completed with B201 antibodies and blotted with B201 and FGFR2 antibodies. (B) HEK293TT cells were transfected with FGFR4 and BPV-1 full-length or E2R (aa 162-410) constructs. BPV-1 E2 protein pull-down was completed with B201 antibodies and blotted with B201 and FGFR4 antibodies. (C) HEK293TT cells were transfected with FGFR1 and BPV-1 full-length or E2R (aa 162-410) constructs. BPV-1 E2 protein pull-down was completed with B201 antibodies and blotted with B201 and FGFR1 antibodies. (D) HEK293TT cells were transfected with FGFR2 and BPV-1 E2 (WT, Y102F, or Y102E) constructs. Pull down was completed with B201 antibodies. Proteins were detected with PY-99, B201, and FGFR2 antibodies.

Fig. 2: FGFR2 impairs BPV-1 transient replication and it is not mediated through Y102. (A) HEK293TT cells (0.5% FBS) were transfected with FGFR1, FGFR2, and FGFR4 constructs. 48h later, cells were lysed in 2% SDS, 150 mM NaCl, 10 mM Tris-HCl, pH 8.0. Immunoblots were completed with FGFR1, FGFR2, FGFR4, pTyr-100, and β-actin antibodies. (B) C33A cells (0.5% FBS) were transfected with BPV-1 E1, E2, pFLORI-BPV-1 (Fluc) , pRL (Rluc) constructs in the presence of FGFR1, FGFR2, and FGFR4 expression plasmids. 72 h later, cells were lysed and firefly and renilla luciferase levels were measured using Dual Glo Luciferase reagent. Firefly luciferase levels were normalized to renilla luciferase levels. Values are expressed as mean +/- SEM, n=8. * are p-values < 0.05. (C) C33A cells were transfected with BPV-1 E1, E2 (WT, Y102F, or Y102E), pFLORI-BPV-1 (Fluc) , pRL (Rluc) constructs in the presence of FGFR2. 72 h later, cells were lysed and firefly and renilla luciferase levels were measured using Dual Glo Luciferase reagent. Firefly luciferase levels were normalized to renilla luciferase levels. Values are expressed as mean +/- SEM, n=4. (D) C33A cells (0.5% FBS)

were transfected with BPV-1 E2, FGFR2, and pGL2-E2BS-Luc. 48 h later, cells were lysed and firefly luciferase levels were measured using Dual Glo Luciferase reagent. Values are expressed as mean +/- SEM, n=4. (E) BPV-1 E2 either by itself or with the FGFR2 kinase was overexpressed in 293TT cells, immunoprecipitated with B201 antibody, and separated by SDS page electrophoresis. The Coomassie stained band corresponding to E2 was excised and analyzed by mass spectrometry for phosphorylated peptides. Tyrosine residues with a high confidence post-translational modification (PTM) score above 75 are summarized here. Homologous residues for HPV-16,-18, and -31 are listed.

Fig. 3: FGFR2 interacts with HPV-31 and HPV-16 E2 proteins. (A) HEK293TT cells were transfected with FLAG-HPV-16 E2, MYC-FGFR1, MYC-FGFR2, and MYC-FGFR4 constructs. FLAG-HPV-16 E2 protein pull-down was completed with M2 antibodies and proteins were detected with M2 and MYC antibodies. (B) The immune complexes of HPV-16 E2 from the W12 cells were analyzed for the presence of endogenous FGFR2, FGFR1 or FGFR4 using specific FGFR antibodies. Normal sheep IgG served as the negative control. HPV-16 E2 protein was detected with TVG-261 antibodies. (C) HEK293TT cells were transfected with FLAG-HPV-31 E2, MYC-FGFR1, MYC-FGFR2, MYC-FGFR3 and MYC-FGFR4 constructs. FLAG-HPV-31 E2 protein pull-down was completed with M2 antibodies and proteins were detected with M2 and MYC antibodies. (D) CIN612 cells were transfected with FLAG-HPV-31 E2. NOKS cells were transfected with FLAG-HPV-31 E2 and FGFR2 constructs. PLA was completed with M2 (mouse) and FGFR2 (rabbit) antibodies. Red foci are an indication that the FLAG and FGFR2 proteins are within close proximity of each other. Foci formation (Red) and DAPI (blue). Each + indicates number of foci observed in the nuclei. (E) HEK293TT cells were transfected with FGFR2 and FLAG-HPV-31 E2. 48 h later, cells were lysed and pull down was completed with M2 beads. Proteins were detected with M2, FGFR2 and p-100 Tyr antibodies.

Fig. 4: FGFR2 impairs HPV-31 transient replication. (A) C33A cells were transfected with pFLORI31 (Fluc) , pRL (Rluc), HPV-31 E1 and E2 constructs in the presence of FGFR2 in 0.5% FBS. 72 h later, cells were lysed and firefly and renilla luciferase levels were measured using Dual Glo Luciferase reagent. Firefly luciferase levels were normalized to renilla luciferase levels. Values are expressed as mean +/- SEM, n=8. * are p-values < 0.05. (B) HEK293TT cells (0.5% FBS) were transfected with pCI, FLAG-HPV-31 E1, FLAG-HPV-31 E2 and FGFR2 constructs. 48 h later, cells were lysed in 2% SDS, 150 mM NaCl, 10 mM Tris-HCl, pH 8.0. Immunoblots were completed with FGFR2, M2, HPV-16 E1, and β-actin antibodies.